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Effect of salinity stress on the antioxidant defence systems of two varieties of cowpea (*Vigna unguiculata L.*)

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Abstract

Osmotic stress, oxidative stress and oxidation of essential macromolecules are common consequences of salinity stress that limit plant growth and productivity. Plants are known to evolve several strategies such as upsurge of antioxidant defence systems (ADS) and accumulation of osmolytes, so as to thrive under such conditions. In the present study, the effect of salinity stress (using irrigation method) on ADS of two cultivars (IT-99 and IT-288) of cowpea was examined. Plant samples (roots, young leaves and matured leaves) were harvested on day 21 of treatment with saline solution (100 – 400 mM NaCl). Antioxidant markers and osmolytes levels were quantified and compared with the controls (0.0 mM NaCl). The activities of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase significantly increased ($p<0.05$) in the leaves, except for IT-288 where catalase activity significantly decreased ($p<0.05$) when compared to the control. On the contrary, catalase and peroxidase activities significantly decreased ($p<0.05$) in the roots of both cultivars. Largely, ascorbate, glutathione (GSH) and tocopherols levels increased as salinity increases, except for GSH in roots of IT-99, and leaves of IT-288. The amount of flavonoids detected in the same tissue were not significantly ($p>0.05$) different in all the salinity levels investigated. The level of proline increased at moderate salinity levels in all samples and at high salinity in roots of IT-99 and mature leaves of IT-288. For IT-99, levels of glycinebetaine significantly increased ($p<0.05$) at high salinity, but significantly decreased at similar levels in IT-288. H_2O_2 levels significantly increased in the roots but decreased ($p<0.05$) in leaves samples. Malondialdehyde concentration generally increased significantly ($p<0.05$) when compared with control. The findings of these study suggest that both cultivars were induced to express higher antioxidant activity and to a certain extent synthesis of more osmolytes.

Keywords: Cowpea, Salinity Stress, Antioxidant Defence System, Osmolytes.

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INTRODUCTION

Cowpea (*Vigna unguiculata*L. Walp.) is an annual leguminous crop commonly referred to as the black-eyed pea (Hadi*et al*., 2012).The plant is widely cultivated in Sub-Saharan Africa especially in the Sahel region of West Africa, this is especially due to its nutritional composition, nitrogen fixing capability, ability to thrive in seemingly dry climates and poor soils (Singh *et al*., 1997).In addition to B vitamins (especially vitamin B9), cowpea is generally rich in protein (about 20%); hus serving as cheap fortification and alternative protein source among poor families and pregnant women (Giami*et al*., 2001; Phillips *et al*., 2003; Rangel *et al*., 2005) For these reasons, the crop is widely cultivated in northern Nigeria were rainfall is short lived, dry season is long, the soil is sandy and poverty among the populace is high (Hadi *et al*., 2012).

Salinity stress is an important abiotic factor that limits the growth of plants. It is reported to induce oxidative stress via ionic toxicity, osmotic stress and generation of reactive oxygen species (Ashraf, 2009; Saidi *et al*., 2010; Chawla *et al*., 2013). More so, salinity stress can result to impaired photosynthesis, dysfunction in electron transport and stomata, DNA damage, lipid peroxidation and oxidation of proteins (Wang *et al*., 2009; Abreu *et al*., 2013; Deinlein *et al*., 2014). At different developmental stages, plants are observed to evolve different mechanisms triggered by salinity and/or other factors so as to thrive (Chen and Murara, 2002; Mahajan and Tuteja, 2005; Hamed *et al*., 2013). Maintaining high antioxidant capacity so as to mop-up or detoxify ROS is an integral subset of these mechanisms (Chen *et al*., 2010). Important among these defence mechanisms is inducing the synthesis of antioxidant enzymes such as SOD, CAT, APX, POX, glutathione reductase and so on (Mittova *et al*., 2004; Zaefyzadeh *et al*., 2009; Gupta and Huang, 2014; Talbi *et al*., 2015).

The cultivation of the crop is accompanied by several challenges that can affect optimal yield. Salinity plays important role on germination, thriving and yield of plants (Ashraf, 2009; Wang *et al*., 2009; Saidi*et al*., 2010). Though cowpea is known to grow well in arid regions with sandy soil having less than 2% humus, variation in salinity is reported to affect the plant's yield. High soil salinity result to both reduced water absorption and ionic imbalance. These eventually result to salinity stress, which is accompanied by oxidative stress, the generation of reactive oxygen species (ROS) among other biochemical and molecular derangements. Consequently, these affect cultivation and overall yield (Ambede *et al*., 2012; Abreu *et al*., 2013; Chawla *et al*., 2013; Deinlein *et al*., 2014). Therefore, understanding the effect of salinity on oxidative stress marker with the aim of establishing tolerance levels and developing salinity tolerant varieties cannot be over emphasised. This study was aimed at investigating the effect of salinity stress on antioxidant defence responses of IT-99 and IT-288 cowpea cultivars.

MATERIALS AND METHODS

Sample collection

Two cultivars of cowpea seeds (IT-99 and IT-288) were obtained from Sokoto Agricultural Development Project (SADP), Sokoto State. The seeds were transported to the Botanical Garden of the Department of Biological Sciences, Usmanu Danfodiyo University, and Sokoto for planting.

Planting and Soil Condition

The cultivars of cowpea seeds were sown on 85 % sandy soil whose characteristics were as follows: pH 6.34, 0.64 % carbon, 0.060 kg nitrate-nitrogen, 0.39 kg sodium, 0.82 kg potassium, 5.8 kg cation exchange capacity, 0.45 kg calcium and 0.50 kg magnesium.

Salt Treatment and Harvesting

The plants were irrigated every three days with sodium chloride solutions (0, 100, 200, 300, and 400 mM). Plants administered solely water (0.0m NaCl solution) served as control. Each treatment was done in triplicate. On Day 21 of the experiment, roots, matured leaf-pair and young (distal) leaf-pair were harvested for analysis.

Assays for Enzyme Activities

Freshly collected samples (0.5g) were individually washed with distilled water, then homogenised for 5 min in 3 ml of ice-cold phosphate buffer (100 mM, pH 7.6) containing 0.1 mM EDTA. The filtrate obtained was centrifuged at 3000 rpm for 15 min. The supernatant obtained was employed in enzyme assay experiments. Standard methods were employed for the estimation superoxide dismutase (SOD) activity (Velikova *et al*., 2000), peroxidase (POX) activity, Catalase (CAT) Activity (Aebi, 1984) and Ascorbate Peroxidase (APX) activity (Gupta *et al*., 1995).

Determination of Non-Enzymatic Antioxidant Parameters

Non-enzymatic antioxidant parameters were determined using 0.5 g of the fresh plant samples. The parameters determined are flavonoids content (Bonham and Kocipai-Abzan, 1994) ascorbic acid (Rutkowski and Grzegorczyk, 2007), reduced glutathione (GSH) concentration (Beutler, 1963)

and tocopherol concentration (Rutkowski and Grzegorczyk, 2007).

Determination of Osmoprotectants

The contents of proline and glycinebetaine in 0.5 g of the samples were estimated according to established methods (Bates *et al*., 1973; Grieve and Grattan, 1983).

Determination of Lipid Peroxidation and Hydrogen Peroxide Levels

From 0.5 g of the plant samples, malondialdehyde as a marker of lipid peroxidation was estimated using thiobarbituric acid method (Hodges *et al*., 1999).On the other hand, hydrogen peroxide content (a causative of lipid peroxidation) was estimated by the standard method (Velikova*et al*., 2014).

Statistical Analysis

The data generatedwere presented as Means ± SEM. The datawere analysed byone-way-analysis of variance (ANOVA) using Graph Pad Instat software (Version 3.0, San Diego, USA). Dunnett Multiple Comparisons Test was also used to compare mean values.

RESULTS AND DISCUSSION

Enzyme Activities

The activity of SOD in the matured leaves of IT-99 cultivar significantly decreased (P<0.05) at 100 mM t NaCl concentration in comparison to the control. However, SOD activity in young leaves increased significantly (P<0.05) at 100 mM salt level, and as well increased significantly (P<0.05) in the root samples at 100 and 200mM NaCl. For young leaves and roots of IT-99, a general decrease in SOD activity was observed with increase NaCl level beyond 100 mM. On the other hand, mature leaves of IT-288 cultivar exhibited significantly diminished (P<0.05) SOD activity at 100 mM NaCl. However, the reduced activity increased progressively when NaCl level increased. The young leaves and roots of IT-288 exhibited a progressive increase in SOD activity as salt concentration increases. Significant increase in SOD activity of this cultivar were observed at (P<0.05) 300 mM NaCl for young leaves, and at 200 and 400 mM for root samples when compared with control. These outcomes indicate that IT-288 cultivar expresses and produces more SOD as the salt levels increases, while the IT-99 was able to do so at around 100 mM NaCl. The decreased SOD activity as salt concentrations is been increased is most likely due to a decline of protein turnover salinity stress increases. An increased SOD signifies the ability of the plants to detoxify accumulating superoxide anion (O_2^-) into H_2O_2 (a less toxic molecule). Therefore, IT-288 cultivar which exhibited an increasing SOD activity as salt concentration increases may be a better/ more resistant to salinity stress compared to the IT-99 cultivar. Plants have been suggested to enhance their SOD activity when exposed to abiotic factors such as drought, metals toxicity and other environmental stresses. Though the level of SOD may decline with increasing stress due to reduced protein turn over, it's over production is positively correlated to increase oxidative stress tolerance (Sharma and Dubey, 2005;Noctor and Foyer, 1998). Previous study indicated that salt tolerant rice possessed an increased SOD activity (Parida *et al*., 2004). In general, SOD is among the first line of defence against reactive oxygen species (Radwan *et al*., 2010).

The POX activity in the matured and young leaves of both IT-99 and IT-288 cultivars increased significantly (P<0.05) at high salt concentrations. Conversely, the activity of this enzyme decreases upon increasing salt level in both IT-99 and IT-288. The increased POX level in leaves may be an adaptation strategy by the plant to detoxify accumulating levels H_2O_2 (produced by SOD) and lipid peroxides among other peroxides to water and lesser reactive/radical molecules. The increasing activity of POX in leaves of the two cowpea cultivars is an indication of their potentials to neutralise peroxide, halt chain reactions and as well protect essential molecules especially in the leaves- an organ where photosynthesis and respiration (gas exchange) is localised.

The CAT activities of the mature and young leaves of IT-99 as well as mature leaves of IT-288 cultivar were observed to increase when salt concentrations increased. Significant increase (P<0.05) was observed at all the tested salt concentrationsforIT-99 matured leaves, only at 200 mM NaCl for IT-99 young leaves, and at both 300 and 400 mM NaCl for IT-288 matured leaves. The CAT activities of both the mature and young leaves of IT-99 were optimal at 200 mM NaCl, whereas optimal for mature leaves of IT-288 at 300 mM NaCl. The CAT activity progressively declined beyond these optimal NaCl levels. In contrary, a significantly diminished (P<0.05) CAT activity in the root of both cultivars (at all tested concentration) and that of IT-288 young leaves (beyond 100 mM NaCl) was detected as compared to controls. Notably, the decline was progressive in roots of IT-99 and young leaves of IT-288, but not in roots of IT-288 cultivar. The decline in CAT activity in leaves of IT-99 cultivar may be consequence of reduced protein turnover. However, the leaves of IT-99 and only matured leaves of IT-288 cultivar exhibited possible salinity tolerance via expressing higher CAT activities. CAT has been reported to be chiefly available in leaves as a result of its richness in

chloroplast, mitochondria and cytosolic components. This enzyme detoxifies the H_2O_2 produced via SOD catalysed reaction to water and oxygen while utilizing no energy (Mallick and Mohn, 2000), increased generation of H_2O_2 is usually coupled to increased activity/expression of CAT in plants (especially in the leaves). However, the expression of the enzyme depends on type and intensity of the stress, overall protein synthesis and the type of plant strain/cultivar (Moussa and Abdel-Aziz, 2008). The young leaves of IT-99 and the matured leaves of both IT-99 and IT-288 cultivars exhibited an increased CAT activity, suggesting their potential to withstand stress and detoxify H_2O_2 generated in that circumstance. Previous study indicated that salt tolerance by tomatoes and citrus is attributed to increase activities of both SOD and CAT (Mittova *et al*., 2004).

APX activity of both cultivars generally increased upon salt treatment in all the samples except for young leaves of IT-288 cultivar. In comparison to controls, significant increase ($P<0.05$) in APX activity was observed at 300 mM NaCl for both young leaves and roots of IT-99, at 100 and 200 mM NaCl for matured leaves of IT-288, at 200mM for young leaves of IT-288 and at both 200 and 300 mM for roots of IT-288 cultivar. Though a decline in APX activity was generally observed at 400 mM salt concentration when compared to other salt treatment (excluding control), the only significant decrease was observed at 300 mM NaCl in young leaves of IT-288 cultivar. APX is an integral part of glutathione-ascorbic acid cycle. It is the most common antioxidant enzyme in plant cells and has higher affinity to H_2O_2 than CAT (Noctor and Foyer, 1998; Noctor *et al*., 2002). These confer to it a vital role in regulating intracellular ROS concentration, especially in the cytosol, stromal cells, thylakoidal of chloroplast, mitochondrial and peroxisomes (Madhusudhan *et al*., 2003; Guan *et al*., 2009). The H_2O_2 generated/accumulated by activity of other enzymes are either scavenged (in organelles) or cleansed/eliminate (in apoplast and cytoplasm) by this enzyme (Madhusudhan *et al*., 2003). APX activity is believed to rise in response to salinity, draught, snow/cold, metal toxicity, irradiation among other abiotic factors (Lopez-Huertas *et al*., 2003). It was indicated that APX was among the enzymes whose expression and activity was increased in salinity resistant strains of tomato and citrus (Mittova *et al*., 2004).

Non-Enzymatic Antioxidant Parameters

In addition to enzymatic antioxidant mechanisms, non-enzymatic parameters such as ascorbate, carotenoids, reduced glutathione (GSH), phenols, flavonoids and tocopherols play significant role in detoxifying/mopping up excess oxidant, protecting vital macromolecules as well as halting chain reactions (Munne, 2005; Agati*et al*., 2012; Rai *et al*., 2013; Gupta and Huang, 2014; Talbi *et al*., 2015). When exposed to abiotic stresses, the synthesis of non-enzymatic compounds is induced, thus leading to elevated levels of the compounds in the plant parts. Findings suggest that plants strains having low non-enzymatic antioxidant levels are relatively more susceptible and sensitive to stress (Yeh *et al*., 2007). The levels of flavonoid, ascorbic acid, GSH and tocopherol are displayed in Table 2. In comparison to the control groups, flavonoid levels were found to increase in both young and matured leaves of the two cultivars. The changes in flavonoid levels of the roots were not significant ($P>0.05$) for all the tested samples when compared to the controls. Since flavonoids act as antioxidants phytochemicals, their increased levels in the leaves of both IT-99 and IT-288 cultivars confers to them a possible resistance to antioxidant stress induced by salinity stress.

In comparison to controls, the concentration of ascorbic acid in matured and young leaves of both cultivars increased significantly at all NaCl concentrations (except for young leaves of IT-288 at 400 mM NaCl, where a significant decrease was detected). However, significant decrease in ascorbic acid was found in roots of IT-99 (at 100 and 200 mM NaCl) and that of IT-288 cultivar (100 mM NaCl). Moreover, higher levels of NaCl (i.e. 300 and 400 mM) resulted to a significant increase of ascorbic acid concentration in both IT-99 and IT-288 cultivar. In comparison to controls, outcomes of the present study indicate an increase in ascorbic acid concentrations in leaves, and a decrease at the roots when salinity was increased. This shows that the leaves (which are the organs for photosynthesis, respiration and synthesis of important macromolecules) were induced to synthesize more ascorbic acid as a mechanism to withstand salinity stress (Noctor*et al*., 2002). These findings are similar to previous reports indicating the ability of plants such as *Arabidopsis thaliana* to express increased ascorbic acid levels when exposed to oxidative stress (Wang *et al*., 2010). Similarly, it was demonstrated that exogenously administered ascorbic acid to tomato seedling dramatically reduced the level of oxidative damage to the plant (Shalata and Neumann, 2001).

Salinity stress was found to significantly increase the GSH level of both matured and young leaves of IT-99 (except for IT-99 at 400 mM NaCl) as compared to the control. At 100 mM NaCl, non-significant increases ($P>0.05$) of GSH levels were noticed in the matured and young leaves of IT-288. In contrary, IT-288 leaves exhibited significantly decreased GSH levels beyond 100 mM NaCl when compared to the controls. The roots of IT-99 exhibited significantly decreased ($P<0.05$) GSH levels as salinity stress increased. Except at 400 mM NaCl, roots of IT-288 showed significantly increased GSH levels as salinity stress increased.

GSH is among the most important antioxidant in all organisms. It prevents and protects against oxidative damage caused by reactive oxygen species and heavy metals halt peroxidation chain reactions and reduce plant stress via GSH-ascorbate cycle among other mechanisms (Ha *et al*., 1999; Noctor *et al*., 2002). The findings of this study are similar to previous reports (Wang *et al*., 2014) in which greater levels of GSH were observed in the chloroplasts of rice after exposure to salinity stress.

In matured and young leaves of both IT-99 and IT288 cultivars, tocopherol levels were found to significantly increase ($p<0.05$) at all NaCl treatment concentrations when compared with control. Additionally, the GSH levels of IT-99 roots significantly decreased at 100 mM NaCl, but significantly increased ($P<0.05$) at 200 and 300 mM NaCl. In root samples of IT-288 cultivar, tocopherol level progressively and significantly increased ($P<0.05$) as the salinity stress increases. Tocopherol as an antioxidant principally limits lipid peroxidation and attenuates chain reaction via converting a peroxyl radical to its corresponding hydrogen peroxide (Maeda *et al*., 2005).

The general increase in tocopherol levels especially at higher salinity level exhibited by the tow cultivars in this study demonstrate the ability of the plants to moderate lipid peroxidation under salinity and/or oxidative stress. In line with our findings, some studies demonstrated that rice strains with tocopherol deficiency had lesser salinity tolerance (Abbasi *et al*., 2007). Similarly, *Cakile maritime* (a halophyte) expressed higher tocopherol levels than *A*. *thaliana* (a glycophyte) upon exposure to salinity stress. Hence, cementing the basis of higher salinity tolerance by *C. maritima* in comparison to most glycophyte (Ellouzi*et al*.,2011).

Osmoprotectant Levels

Increased salinity is accompanied by osmotic stress in plants. This could trigger plants to evolved new strategies in order to circumvent the effect of osmotic stress especially in the roots. The osmolytes (e.g., proline and glycinebetaine) that facilitate water absorption by means of decreasing cytoplasmic osmotic potential are indispensable approach of mitigating this type of stress (Hu *et al*., 2913; Ahanger *et al*., 2014' Pottom*et al*., 2014; Puniran-Hartley *et al*., 2014). As seen in Table 3, proline level of IT-99 cultivar increased significantly ($P<0.05$) only at 200mM NaCl in matured leaves and at 100 and 200mM NaCl in young leaves It is worthwhile to mention that a significant decline ($P<0.05$) in proline levels of the leaves (when compared to controls) was realized at higher salinity stress state (300 and 400 mM NaCl). The roots of this cultivar exhibited significantly decreased ($P>0.05$) proline level at 100mM NaCl. However, the proline levels significantly ($P<0.05$) and progressively rise as salinity stress increased. As salinity increases, the matured leaves of IT-288 cultivar showed a significant and progressive increase ($P<0.05$) in proline level. Though significant increases were observed at 100 mM NaCl in young leaves and roots of IT-288, significant decrease in proline levels were observed beyond 100 mM NaCl in young leaves and beyond 200 mM NaCl in the roots when compared with control. This above finding agrees to previous reports that showed an upsurge and accumulation of proline in response to salinity stress (Manjili *et al*., 2012).

More so, the proline concentration in roots of salinity tolerant alfalfa is found to be promptly double upon exposure to increasing salinity (Wanicov and Bastola, 1997). The concentrations of glycinebetaine were observed to significantly increase beyond 200 mM NaCl for both mature and young leaves of IT-99, and only at 400 mM NaCl in the roots. Conversely, at 300 and 400 mM NaCl in matured leaves and at 400 mM NaCl were seen in IT-288 cultivar. The root of this cultivar did not show any significant change in glycinebetaine at all tested salinity levels. The above outcome is supported by previous studies that reported an accumulating level of glycinebetaine in response to stress by crops such as spinach, barley, tomato, potato, rice, carrot and sorghum (Yang *et al*., 2003; Chen and Murata, 2011).

Hydrogen Peroxide and Malondialdehyde (Lipid Peroxidation) level

The levels of hydrogen peroxide and malondialdehyde are also presented in Table 3. At 100 and 400 mM NaCl, the hydrogen peroxide levels of the matured leaves of IT-99 significantly increased. Its young leaves showed a significant increase at 100mM NaCl and a significant decrease beyond the 100 mM salinity level. In the roots, a significantly decreased H_2O_2 levels was seen at 100 mM NaCl, while significantly increased levels were observed at 300 and 400 mM NaCl. For IT-288 cultivar, H_2O_2 level progressively and significantly decreased at all salinity levels in the mature leaves and at 300 and 400 mM in the young leaves. Significant increase was found at 100 and 200 mM NaCl in young leaves as well as at 400 mM NaCl in the roots. The increased level of H_2O_2 in the roots as against its decreased level in leaves is a direct consequence of the inability of the roots to rapidly synthesize both enzymatic and non-enzymatic antioxidants. This is also coupled with the fact that most biosynthetic process, photosynthesis, and gaseous exchange primarily occurs in leaves rather than the roots of plants.A significant increase in malondialdehyde level was generally observed at salinity levels in all the samples of the two cultivars when compared to controls.

Table 1: Enzymatic Activities (Unit/ml) of Two Varieties of Cowpea Exposed to Salinity Stress

Enzyme	NaCl (mM)	Cultivar					
		IT-99			IT-288		
		Mature Leaf	Young Leaf	Root	Mature Leaf	Young Leaf	Root
SOD	0	77.33 ± 15.67	20.93 ± 1.57	12.22 ± 0.18	56.44 ± 18.83	2.28 ± 0.04	25.11 ± 2.61
	100	19.37 ± 1.57*	77.33 ± 15.67*	34.31 ± 0.15*	3.62 ± 0.08*	4.32 ± 0.10	35.55 ± 5.22
	200	61.60 ± 15.70	40.77 ± 5.22	15.44 ± 0.43*	8.75 ± 0.35	8.00 ± 0.45	75.55 ± 14.78*
	300	72.11 ± 20.89	9.51 ± 0.04	13.05 ± 0.09	40.77 ± 5.22	56.44 ± 18.83*	60.77 ± 14.78
	400	32.49 ± 6.91	9.26 ± 018	11.88 ± 0.17	56.44 ± 18.83	20.93 ± 1.57	90.33 ± 4.91*
POX	0	3.33 ± 0.03	0.53 ± 0.01	4.73 ± 0.02	4.13 ± 0.02	4.03 ± 0.03	5.33 ± 0.01
	100	3.44 ± 0.02	3.34 ± 0.04*	2.96 ± 0.02*	10.70 ± 0.03*	4.20 ± 0.04*	4.53 ± 0.02*
	200	5.72 ± 0.04*	9.33 ± 0.04*	3.04 ± 0.02*	6.53 ± 0.02*	8.18 ± 0.01*	4.00 ± 0.01*
	300	6.29 ± 0.08*	12.65 ± 0.04*	2.90 ± 0.01*	4.76 ± 0.02*	12.61 ± 0.05*	3.50 ± 0.01*
	400	6.53 ± 0.02*	6.43 ± 0.04*	2.58 ± 0.03*	4.30 ± 0.01*	6.49 ± 0.02*	3.34 ± 0.04*
CAT	0	1247.40 ± 15.21	1852.80 ± 63.89	2172.20 ± 127.78	2252.10 ± 47.92	1672.20 ± 52.27	2418.00 ± 117.95
	100	1522.70 ± 22.73*	2418.00 ± 117.95	1852.80 ± 63.89*	1672.20 ± 52.27	1597.70 ± 66.61	1545.50 ± 22.73*
	200	2535.90 ±117.95*	2875.00 ± 331.98*	1482.70 ± 55.01*	3291.40 ± 158.62	1420.00 ± 40.00*	1177.40 ± 27.00*
	300	1991.80 ± 37.58*	2535.90 ± 117.95	1192.60 ± 42.59*	9269.70 ± 1184.90*	1262.60 ± 15.21*	1247.40 ± 15.21*
	400	1620.50 ± 52.27*	2209.80 ± 90.19	1005.60 ± 19.91*	5402.00 ± 801.96*	1126.00 ± 23.96*	1345.90 ± 34.07*
APX ($\times 10^{-4}$)	0	1.16 ± 0.33	0.53 ± 0.15	0.30 ± 0.00	1.00 ± 0.25	3.07 ± 0.59	1.33 ± 0.27
	100	1.33 ± 0.52	1.53 ± 0.83	0.83 ± 0.35	4.00 ± 0.60*	1.23 ± 0.32	1.33 ± 0.35
	200	2.33 ± 0.41	1.60 ± 0.10	1.73 ± 0.61	2.23 ± 0.79	6.86 ± 0.23*	6.90 ± 2.55*
	300	1.70 ± 0.70	2.27 ± 0.27*	2.20 ± 0.31*	4.00 ± 0.60*	1.00 ± 0.60*	13.47 ± 0.14*
	400	1.57 ± 0.72	0.87 ± 0.23	1.73 ± 0.65	1.73 ± 1.13	1.33 ± 0.49	1.27 ± 0.27

Values are expressed as Mean ± SEM of three replicates.
* = Significant difference ($P<0.05$) when compared to control group (column) using Dunnett Multiple Comparisons Test.

Table 2: Concentration of Non-Enzymatic Antioxidants of Two Varieties of Cowpea Exposed to Salinity Stress

Parameters	NaCl (mM)	Cultivar					
		IT-99			IT-288		
		Mature Leaf	Young Leaf	Root	Mature Leaf	Young Leaf	Root
Flavonoid (mg/100g)	0	16.67 ± 3.33	13.33 ± 3.33	13.33 ± 3.33	26.67 ± 3.33	26.67 ± 6.67	16.67 ± 3.33
	100	33.33 ± 14.53	30.00 ± 5.77	10.00 ± 0.00	43.33 ± 6.67	26.67 ± 8.82	13.33 ± 3.33
	200	40.00 ± 11.55	16.67 ± 6.67	10.00 ± 0.00	46.67 ± 6.67	26.67 ± 3.33	10.00 ± 0.00
	300	33.33 ± 6.67	20.00 ± 0.00	10.00 ± 0.00	33.33 ± 3.33	36.67 ± 3.33	10.00 ± 0.00
	400	26.67 ± 6.67	16.67 ± 3.33	10.00 ± 0.00	23.33 ± 3.33	40.00 ± 0.00	10.00 ± 0.00
Ascorbate (µg/g)	0	321.22 ± 4.08	374.74 ± 1.73	346.02 ± 1.73	238.95 ± 1.13	373.45 ± 0.65	267.68 ± 1.73
	100	333.58 ± 32.68	480.51 ± 3.38*	218.06 ± 0.65*	374.75 ± 0.65*	376.06 ± 1.13	259.19 ± 1.30*
	200	566.69 ± 1.73*	489.00 ± 1.26*	248.74 ± 1.13*	413.53 ± 3.63*	411.97 ± 2.85*	270.29 ± 0.00
	300	728.61 ± 1.96*	714.90 ± 1.12*	470.07 ± 1.13*	552.33 ± 1.13*	396.95 ± 0.65*	629.37 ± 0.65*
	400	677.89 ± 10.99*	667.89 ± 1.12*	569.96 ± 1.13*	573.87 ± 1.13*	323.17 ± 1.13*	650.91 ± 1.73*
GSH (µg/g)	0	21.01 ± 0.42	21.26 ± 0.25	34.31 ± 0.15	37.88 ± 2.62	22.68 ± 0.30	1.11 ± 0.19
	100	39.10 ± 0.42*	39.30 ± 0.69*	15.44 ± 0.43*	41.79 ± 0.31	23.85 ± 0.24	19.60 ± 0.34*
	200	27.17 ± 0.27*	35.94 ± 0.07*	13.05 ± 0.09*	25.51 ± 0.37*	19.55 ± 0.13*	9.48 ± 0.34*
	300	22.38 ± 0.24*	27.13 ± 0.34*	12.22 ± 0.18*	12.51 ± 0.30*	15.35 ± 0.34*	2.39 ± 0.18*
	400	21.89 ± 0.13	24.14 ± 0.13*	11.88 ± 0.17*	13.49 ± 0.09*	13.73 ± 0.55*	1.80 ± 0.18
Tocopherol (µg/g)	0	62.70 ± 0.45	92.29 ± 0.16	81.69 ± 0.26	51.24 ± 0.36	70.49 ± 0.20	48.11 ± 0.12
	100	65.78 ± 0.46*	82.57 ± 0.16*	68.49 ± 0.16*	54.83 ± 0.15*	69.26 ± 0.37	49.35 ± 0.16*
	200	82.45 ± 0.42*	103.53 ± 0.10*	97.76 ± 0.50*	66.20 ± 0.72*	74.97 ± 0.36*	61.19 ± 0.16*
	300	89.81 ± 0.31*	117.90 ± 0.16*	103.24 ± 0.31*	80.09 ± 0.33*	109.63 ± 0.36*	62.19 ± 0.18*
	400	104.46 ± 0.13*	123.02 ± 0.21*	81.68 ± 0.24	113.19 ± 0.16*	97.94 ± 0.42*	67.61 ± 0.16*

Values are expressed as Mean ± SEM of three replicates.
* = Significant difference (P<0.05) when compared to control group (column) using Dunnett Multiple Comparisons Test.

Table 3: Levels of Osmoprotectants (Proline and Glycinebetaine), Malondialdehyde and Hydrogen Peroxide of Two Varieties of Cowpea Exposed to Salinity Stress

Parameters	NaCl (mM)	Cultivar					
		IT-99			IT-288		
		Mature Leaf	Young Leaf	Root	Mature Leaf	Young Leaf	Root
Proline (µg/g)	0	1.97 ± 0.04	1.84 ± 0.01	2.00 ± 0.01	1.93 ± 0.01	2.07 ± 0.01	2.22 ± 0.01
	100	1.90 ± 0.01	1.91 ± 0.01*	1.92 ± 0.01*	2.40 ± 0.01*	2.40 ± 0.01*	2.63 ± 0.00*
	200	2.24 ± 0.01*	2.00 ± 0.01*	2.36 ± 0.00*	2.26 ± 0.01*	1.97 ± 0.01*	2.24 ± 0.01
	300	1.89 ± 0.00*	1.56 ± 0.01*	2.59 ± 0.01*	2.45 ± 0.00*	1.95 ± 0.01*	1.46 ± 0.01*
	400	1.74± 0.01*	1.57 ± 0.01*	2.61 ± 0.01*	2.62 ± 0.00*	1.73 ± 0.00*	1.56 ± 0.00*
Glycinebetaine (µg/g)	0	147.00 ± 1.16	145.00 ± 0.58	151.67 ± 1.20	152.00 ± 0.57	152.67 ± 0.67	152.00 ± 0.58
	100	147.33 ± 0.88	147.33 ± 0.88	152.33 ± 0.88	153.33 ± 0.88	153.33 ± 0.88	151.33 ± 0.88
	200	149.33 ± 0.88	147.33 ± 0.88	153.00 ± 0.58	150.00 ± 1.00	150.67 ± 0.88	151.67 ± 0.33
	300	151.67 ± 0.33*	151.67 ± 0.67*	154.00 ± 0.58	146.00 ± 0.58*	150.33 ± 0.33	152.67 ± 0.88
	400	157.67 ± 0.67*	156.00 ± 0.58*	155.00 ± 0.00*	142.33 ± 0.88*	147.00 ± 0.58*	154.00 ± 1.16
Hydrogen Peroxide (µg/g)	0	7.33 ± 0.88	23.00 ± 1.16	11.67 ± 0.33	56.00 ± 1.16	42.33 ± 0.88	39.67 ± 0.33
	100	17.67 ± 0.88*	36.33 ± 0.33*	7.67 ± 0.88*	51.67 ± 1.20*	51.67 ± 1.20*	37.00 ± 0.58
	200	4.33 ± 0.88	17.00 ± 0.58*	8.67 ± 0.33	44.00 ± 1.16*	48.33 ± 0.67*	38.00 ± 1.16
	300	9.33 ± 0.33	11.00 ± 0.58*	19.00 ± 1.53*	37.33 ± 0.33*	36.67 ± 0.33*	42.67 ± 0.88
	400	13.00 ± 0.58*	11.00 ± 0.58*	22.67 ± 0.88*	35.00 ± 0.58*	36.00 ± 0.58*	48.00 ± 0.58*
Malondialdehyde (µg/g)	0	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.03 ± 0.01
	100	0.51 ± 0.00*	0.18 ± 0.01*	0.48 ± 0.01*	0.10 ± 0.01*	0.22 ± 0.01*	0.30 ± 0.01*
	200	0.53 ± 0.01*	0.42 ± 0.01*	0.52 ± 0.01*	0.59 ± 0.00*	0.41 ± 0.00*	0.79 ± 0.01*
	300	0.62 ± 0.01*	0.79 ± 0.01*	0.56 ± 0.01*	0.62 ± 0.01*	0.39 ± 0.04*	1.26 ± 0.01*
	400	0.65 ± 0.01*	0.85 ± 0.01*	0.70 ± 0.01*	0.58 ± 0.01*	0.46 ± 0.00*	1.29 ± 0.01*

Values are expressed as Mean ± SEM of three replicates.
* = Significant difference (P<0.05) when compared to control group (column) using Dunnett Multiple Comparisons Test.

The increasing level of malondialdehyde was positively correlated to the level of salinity stress. Thus, suggesting increasing levels of peroxidation as salinity and osmotic stress increased. Generally increased level of malondialdehyde in all the tested samples is a direct consequence of lipid oxidation due to oxidative stress. This finding is in accordance with the observed accumulated levels of malondialdehyde in stress exposed plants. ROS scavengers were up-regulated in *Physcomitrella patens* suggesting that the antioxidative system could play a vital role in protecting cells from oxidative damage following exposure to salinity stress (Wang *et al*., 2009).

CONCLUSION

This study revealed the antioxidants system invoked by young leaves, matured leaves and roots IT-99 and IT-288 cultivars under salt stress comprise of enzymatic and non-enzymatic components. Nevertheless, the antioxidant system of the plants as well as synthesis of osmolytes was induced and the level of this protected was relatively dependent on the extent of salinity stress. Thus, suggesting that both cultivars are responsive to salinity stress and may thrive in soil containing moderate salt levels (200 – 300 mM NaCl) or irrigation water with moderate salt concentration. Further study should be carried out morphological parameters and gene expression study.

Conflicts of Interest

The authors have no conflict of interest to declare.

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